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OBSERVATIONS ON PARATYPHOID BACILLI RECENTLY ISOLATED FROM ANIMALS

Robb Spalding Spray

From Purdue University, Agricultural Experiment Station, Lafayette, Ind.

In the recent study of an outbreak of highly infectious enteritis among swine a number of strains of the paratyphoid-enteritidis group were isolated, which offer excellent material for comparison with the reports of other investigators. Most of these strains conform to the standards at present accepted as diagnostic of the various groups, but there are numerous significant departures from these standards.

It is not the purpose to increase the now diminishing confusion in this intricate group, but to record certain striking deviations from type among recently isolated strains. That these variations are not to be regarded as resulting from conditions of artificial culture is evident by the fact that they appeared immediately on isolation and have been maintained through a number of generations. If the variations were limited to cultural characteristics they might be disregarded, but they are in all cases correlated with variations in agglutination.

In addition to the strains isolated from diseased swine, several others were isolated from hog cholera virus blood, in the course of routine serum production. These have been included for the purpose of comparison with the other strains.

Sources and Methods of Isolation

From Diarrheal Swine.—Routine inoculations were made from heart blood, lung, liver, spleen, kidney, and mesenteric glands. The identity of all paratyphoid strains isolated from the various organs of the same carcass may not be assumed, because in at least three instances two, or even three, distinct strains were isolated from the same body.

From Hog Cholera Virus.—(1) By Direct Plating: Samples of all lots of virus blood being tested for gas-producing bacteria by inoculation into glucose broth fermentation tubes. In case gas-formers were present a loop of the same blood was plated in litmus-lactose agar, and in most cases pure cultures of paratyphoids were obtained.

(2) From Rabbits: Whenever a loop of blood was plated a rabbit was inoculated subcutaneously with 0.25 c c of the same blood. When the plate showed paratyphoids the corresponding rabbit usually died within 14 days. Inoculations were made from heart, lung, liver, spleen, and kidney, and the resulting cultures compared, and all but one of a series of apparent duplicates discarded.

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The remaining culture was replated three times to insure purity. From the third plate two colonies were picked and grown on agar slants. At the end of 24 hours these two cultures were inoculated into glucose and lactose broth fermentation tubes, nutrient broth, milk, gelatin, and peptone water. All of the strains included in this paper displayed the accepted paratyphoid-enteritidis reactions in these mediums. If the two strains from the same source gave identical reactions one was discarded and the remaining one became the stock strain. From this culture the group determination was carried out by inoculation of a number of diagnostic mediums. Strains isolated from rabbits were compared with corresponding strains isolated by direct plating, When duplicates were evident the strain isolated by plating was retained and that from the rabbit discarded. Only when there was an evident difference were two strains kept from the same blood sample.

With few exceptions the strains were readily classified. Several, however, displayed such crossing of characters that it was impossible to determine their proper relative position, based on morphologic and cultural characteristics alone. These atypical strains were then used as a basis for classification on the evidence of agglutination and absorption tests.

Twenty-three of the strains discussed in this paper were isolated from the tissues of diseased swine; 8 by plating of hog cholera virus blood; 9 from rabbits that died following inoculation with virus blood known to contain gas-producing bacteria.

Included for comparison only, are two strains of B. paratyphosus A, two of B. paratyphosus B, and one of B. enteritidis, all of human origin.

Table 1 gives the essential diagnostic cultural reactions.

TABLE 1

ESSENTIAL DIAGNOSTIC CULTURAL CHARACTERISTICS

Cultures	Glucose Serum Water	Lead Acetate Agar	Milk Alkaline at	Translucent Opalescent at 90 days	Arabinose	Xylose	Dulcite	Inosite
B. suipestifer 49.....	+	—	4 days	+	— —	+ +	— —	— —
Intermediates....... Rabbit 91 Swine 163	—	+	6 days	+	— —	+ +	— —	— —
B. paratyphosus A Human A97 Swine 134 Virus 149	+	—	35 days	—	+ —	— —	+ +	— —
B. paratyphosus A Human A98	+	+	38 days	—	+ —	— —	+ +	— —
B. paratyphosus B Human B96 Rabbit 124, 135	—	+	3 days	+	+ +	+ +	+ +	+ +
B. enteritidis........ Human 117	—	+	2 days	+	+ +	+ +	+ +	— —

Glucose serum water: + = pink or red; — = decolorized.
Lead acetate agar: + = blackening; — = no black.
Various sugars in serum water: + + = acid, coagulation, and gas; — — = no change; + — = deferred reaction, gas not evident.

Strains from Diarrheal Swine.—Twenty-two of the 23 strains from diseased swine gave cultural reactions which clearly would classify them as B. suipestifer. All produced slight initial acidity in litmus milk in 24 hours, followed by alkalinity on the third to the twelfth day; they produced translucence and opalescence in milk at 35 to 50 days; and they did not blacken lead acetate agar.[1] They did not ferment arabinose, dulcite, nor inosite within 48 hours, but fermented xylose within from 6 to 18 hours, producing acid and gas. They fermented glucose serum water, producing acid and gas, without perceptible reduction of the Andrade indicator.[2]

Several strains showed latent and irregular fermentation of arabinose and dulcite, traces of acid appearing at from 5 to 8 days. In addition, the 22 strains formed a homogeneous agglutinating group.

The single remaining strain, 163, is of particular interest. While it is a B. suipestifer type, this strain showed striking variations immediately on isolation. Inoculation into lead acetate agar produced blackening second to none except that of B. enteritidis 117. Glucose serum water showed acid, gas and coagulation, with strong decolorization at 18 hours; mannite serum water showed decolorization to a degree; arabinose, dulcite and inosite were not fermented; xylose was fermented in 6 hours with the production of acid and gas.

Agglutination tests placed this strain with the other 22 strains. Absorption tests, however, showed it to be a distinct type, intermediate between the B. suipestifer type strain 49, and B. paratyphosus B human type strain B 96.

A strain, 91, discussed later, was isolated from a rabbit inoculated with hog cholera virus blood. This strain showed the same peculiarities as strain 163. Agglutination and absorption tests showed the two to be closely related, if not identical. As this paper is being prepared the same type has been isolated for the third time.

Six strains were tested for pathogenicity against rabbits. All killed in 72 hours when 0.2 c c of a 24-hour broth culture was injected intraperitoneally. The same amount injected subcutaneously on the abdomen caused death in 6 or 7 days. Three attempts to isolate the infecting organism resulted in the recovery of an organism culturally and agglutinatively identical with the strain used for inoculation.

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[1] Jordan and Victorson: Jour. Infect. Dis., 1917, 21, p. 571.

[2] Krumwiede, Kohn and Valentine: Jour. Med. Research, 1919, 39, p. 453.

It is evident that the 23 strains isolated from diseased swine are, in spite of serious deviation from type in a single instance and frequent individual peculiarities, very closely related.

Strains Isolated by Plating of Hog Cholera Virus Blood.—Five of the 8 strains isolated by plating virus blood are typical B. suipestifers in all respects. Two strains, 125 and 146, are atypical only in the irregular fermentation of dulcite.

The single remaining strain, 149, is an "A" type, and is indistinguishable from the two human B. paratyphosus A strains, except as noted later for strain A 98.

Strains Isolated from Rabbits.—Four of the 9 strains isolated from rabbits are typical B. suipestifers in all respects. One strain, 126, is atypical only in the irregular fermentation of dulcite.

Two strains, 124 and 135, are "B" types, and are indistinguishable from the two human B. paratyphosus B strains B 95 and B 96. Conversely, they are sharply distinct from all the B. suipestifer strains, and absorption tests confirm the cultural and agglutination evidence beyond reasonable doubt.

One strain, 134, is an "A" type, and is indistinguishable from the two human B. paratyphosus A strains, except as noted later for A 98.

The single remaining strain, 91, was referred to as being apparently identical with strain 163. This strain, 91, was isolated early in the work, and was at first regarded as a typical B. suipestifer. Prompt blackening of lead acetate agar, and decolorization of glucose serum water, however, showed it to be atypical. Agglutination and absorption tests showed it to be sharply distinct from B. suipestifer 49, and from B. paratyphosus B 96. The same tests clearly indicate its close relationship, if not its identity, with strain 163.

From close observation it appears that these two strains should be regarded as atypical B. suipestifers, and the finding emphasizes the broad extent of possible variation toward the B. paratyphosus B type. It emphasizes further, the contention of Krumwiede[3] that B. suipestifer should be considered not as a "type," but as a "group," wherein wide latitude for variation should be allowed.

Human Strains Used for Comparison.—These five strains are old stock strains, and if variation may be induced by long continued cultivation on artificial mediums, every opportunity has undoubtedly been offered. However, only a single strain, A 98, showed such tendency.

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[3] Personal communication.

Both "A" strains, A 97 and A 98, behaved according to the accepted standards, except that A 98 persistently caused immediate and extreme blackening in lead acetate agar.

Both "B" strains, B 95 and B 96, behaved in all respects according to the accepted standards.

The single B. enteritidis strain was culturally identical with the two "B" strains, except in its inability to ferment inosite, which confirms the observations of Krumwiede[4] and others[5] on the diagnostic value of inosite. Agglutinatively, this strain was distinct from all other strains encountered.

AGGLUTINATION AND ABSORPTION METHODS

Rabbits were used for the production of antiserum. Intravenous injections of 0.5 to 1 c c of a moderately dense suspension of a 24-hour agar culture, heated and cresolized, at three day intervals produced a serum titer of 1:7,500 to 1:54,000.

Twenty-four hour broth cultures, diluted to uniform density with formaldehyd-salt solution, were used as antigen.

The absorption tests were performed by a slight modification of the method of Krumwiede, Kohn, and Valentine.[6] The absorbing cultures were grown on agar in pint whisky flasks. Four flasks were inoculated and incubated for 24 hours.

A stock dilution of the antiserum in salt solution, 1:15, was prepared, and 5 c c used to wash off the growth of the first flask. This washing was transferred to the second flask, and the process repeated for the other two. The four flasks were rinsed with a second 5 c c portion of dilute serum, and the rinsing added to the first washing. About 8 c c of a very dense suspension was recovered.

This suspension was incubated from 2 to 3 hours, then centrifugalized at high speed till clear. It was found that the proportion of sedimented bacteria to serum was between 1:3 and 1:4, and that the agglutinins for the absorbing strain were exhausted or reduced to barely a trace where the serum was of very high titer.

This absorbed antiserum was then preserved with a drop of chloroform, and stored in the refrigerator, where its agglutinating powers were retained for as long as four months.

Agglutinations were carried out by the macroscopic method, and when peculiarities were observed the tests were repeated.

The results of the agglutination tests are summarized in table 2. It was found that all strains culturally B. suipestifer agglutinated identically with the type strain 49, and the record of this strain may be accepted as the summary for the group.

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[4] Jour. Med. Research, 1918, 38, p. 112.

[5] Tenbroeck: Jour. Exper. Med., 1918, 28, p. 764.

[6] Jour. Med. Research, 1918, 38, p. 105.

TABLE 2

HIGHEST SERUM DILUTION SHOWING PERCEPTIBLE AGGLUTINATION

Cultures	Serums				
	B. suipestifer 49	Intermediate 91	B. paratyphosus B B96	B. paratyphosus A A98	B. enteritidis E117
B. suipestifer 49	1:30,000	1:15,000	1:3,000	....	....
Intermediates Rabbit 91 Swine 163	1:15,000	1:30,000	1:7,500	....	....
B. paratyphosus A Human A98 Swine 134 Virus 149	....	....	....	1:15,000	....
B. paratyphosus B Human B96 Rabbit 124, 135	1:3,000	1:3,000	1:15,000	....	....
B. enteritidis Human E117	....	....	....	....	1:7,500

Rabbit strain 91 and swine strain 163 are not only agglutinatively identical, but have affinities for both B. suipestifer and B. paratyphosus B, and should be regarded as intermediates.

The agglutination tests of rabbit strains 124 and 135 confirm the cultural evidence of their identity with human B. paratyphosus B 96. Rabbit strain 134 and virus strain 149 are likewise identical with B. paratyphosus A strain 98.

Absorption tests were set up whenever necessary to clear up the confusion of group agglutination. These results are summarized in table 3. Only the essential ones are recorded.

TABLE 3

ABSORPTION TESTS SHOWING RELATIONSHIP BETWEEN INTERMEDIATES AND B. SUIPESTIFER

Cultures	Serums					
	B. suipestifer 49		Intermediate 91		B. paratyphosus B B96	
	Absorbed by Intermediate 91	Absorbed by B. paratyphosus B B96	Absorbed by B. suipestifer 49	Absorbed by B. paratyphosus B B96	Absorbed by B. suipestifer 49	Absorbed by Intermediate 91
B. suipestifer 49	1:30,000	1:30,000	1:750	1:15,000	....	....
Intermediates Rabbit 91 Swine 163	....	1:15,000	1:7,500	1:30,000	....	....
B. paratyphosus B Human B96 Rabbit 124, 135	....	....	....	....	1:15,000	1:15,000

The record of type strain B. suipestifer 49 may again be accepted as representative of the strains culturally B. suipestifer.

Intermediate strains 91 and 163 are evidently identical and distinct alike from B. suipestifer and B. paratyphosus B. At the same time the absorption of antiserum 91 by B. suipestifer 49 antigen indicates close relationship.

Rabbit strains 124 and 135 are identical with B. paratyphosus B 96.

SUMMARY

An intimate study of the tissues of carcasses of several hundred swine dying of enteric diseases has shown the predominating bacterial flora to be members of the paratyphoid-enteritidis group.

Thirty-four out of 40 strains studied in detail were typical B. suipestifers; two were identical with human B. paratyphosus A; two were identical with human B. paratyphosus B; two were intermediate between B. suipestifer and B. paratyphosus B. B. enteritidis was not encountered at any time.

No true representative of the paratyphoid-enteritidis group has been isolated from feces nor from the lumen of the intestine in a large number of attempts. This fact is very striking in view of the predominance of this group in the tissues.

It has been possible in this work to confirm in all respects the reports of other workers on the value of arabinose, xylose, dulcite, inosite, lead acetate agar, and glucose serum water as diagnostic mediums for the differentiation of the members of the paratyphoid-enteritidis group.